Triple Enzyme Mouse Tumor Digestion

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

Collagenase, type IV
Sigma, Cat. C5138
1 g/100 ml = 10X HBSS Stock
DNase, Type IV
Sigma, Cat. D5025
20,000 Units/100 ml HBSS = 10X Stock
Fungizone Antimycotic
Lyophilized, 20 ml
Invitrogen Corp., Cat. 15295017
Hank's Buffered Salt Solution
HBSS, without Calcium Chloride or Magnesium Chloride
Mediatech, Cat. 21-022-CV (1-800-235-5476)
Hyaluronidase, Type V
Sigma, Cat. H6254
100 mg/100 ml HBSS = 10X Stock
Petri Dishes 100X 15mm
Disposable Sterile Petri Dishes 100X 15mm
Falcon, Cat. 351029; VWR, Cat, 25373-100
70 µm Nylon mesh filter unit
BD Falcon, Cat. 35-2350
50 ml Falcon tube
BD Falcon, Cat. 35-2070

Preparation

10X Triple Enzyme Stock Solution:

Collagenase	1 g	f.c. [10 mg/ml]
Hyaluronidase	100 mg	f.c. [1 mg/ml]
DNase	20,000 Units	f.c. [200 mg/ml]
HBSS	100 ml	

Sterile filter (0.22 µm) and store 5 ml aliquots at –20˚C.
Thaw at **RT** (NOT 37°C) before use.

Establishment of Primary Cultures

1. Remove tumor and place into a 100 mm petri dish and add 5-10 ml HBSS (Hank's balanced salt solution).

2. Quickly mince tumor with scalpels into fragments small enough to be pulled into a 5 ml pipette without getting stuck.

3. Transfer to 50 ml non-vented tissue culture flask.

4. Rinse petri dish with up to 40 ml HBSS and transfer to flask. Total volume in flask should be 45 ml (if not, bring up to volume with HBSS).

5. Add 5 ml 10X Triple Enzyme Mix to the flask, add a stir bar, and incubate at RT on stir plate for 1-3 hours.

6. Pipet up and down to further dissociate cells and pass through 70 μm nylon mesh filter unit into a 50 ml Falcon tube (NOTE: filter unit doesn't fit well into tubes from other companies).

7. Pellet cells at 1200 rpm for 8 min.

8. Wash cells 2-3 x in 14 ml HBSS.

9. Resuspend with appropriate volume of plating media and transfer to culture vessel (6 cm^2 plate or T-25 flask).